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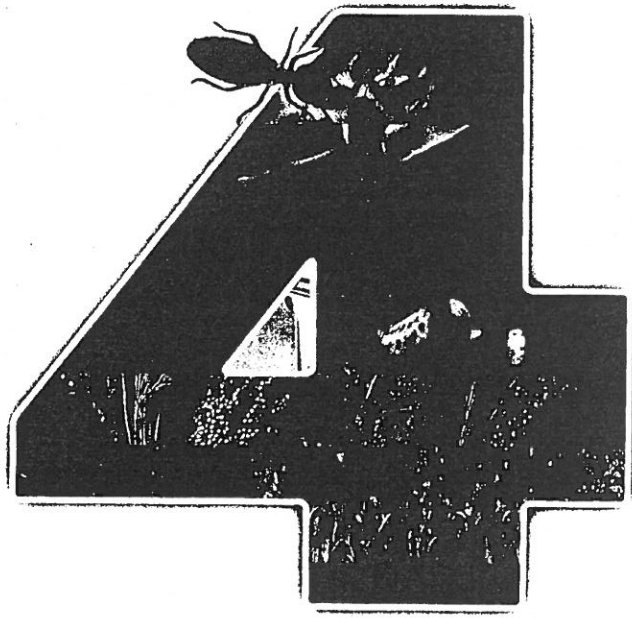

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Rice Cell Wall Ferulic Acid Content Throughout Development

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ABSTRACT

Identification of genes encoding cell wall modifying enzymes has applications in human and animal nutrition, plant defense against pathogens, and biofuel production efficiency. For plants with type II cell walls, such as rice and other grasses, glucuranoarabinoxylans (GAX) are a major component of the hemicellulose found in both primary and secondary walls. Ferulic acid, a phenolic compound, is added to the O-5 of arabinosyl units of GAX. Ferulic acid residues can covalently crosslink arabinosyl residues through dimer formation, or serve as attachment points between GAX chains and lignin, providing rigidity to the cell wall matrix. Therefore, reducing the ferulic acid content of type II cell walls may improve feedstock deconstruction for production of biofuels from bioenergy grasses. The genes that encode feruloyltransferases (FTs) have not yet been identified. However, Mitchell and colleagues (2007) have recently predicted that FTs may be encoded by a clade of genes from the CoA acyl transferase superfamily based on their greater EST abundance in grasses compared with dicots. To attempt to correlate cell wall composition and gene expression across rice development, we are collecting RNA and cell wall samples from various rice organs. In striking contrast to published data for wheat (Obel *et al.* 2002), we observe ferulic and *p*-coumaric acid to be high in young rice tissues. Amounts are also high in stem, node, and other lignified organs. We are now conducting quantitative RT-PCR to test whether any of the putative FT genes identified by Mitchell *et al.* also show this pattern of expression. Any genes from the 20-member family that correlate with ferulic acid accumulation will be targeted for functional studies toward improving the deconstruct-ability of grass cell walls.

WHAT IS GAX?

Grass-specific ferulic acid modifications on glucuranoarabinoxylan (GAX, shown below) crosslink GAX polymers and may serve as sites for lignin integration with polysaccharides. Reduction of grass wall FA content may increase feedstock deconstructability.

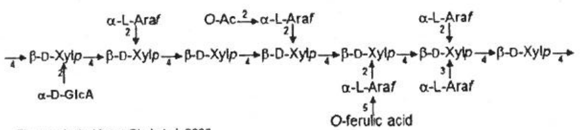
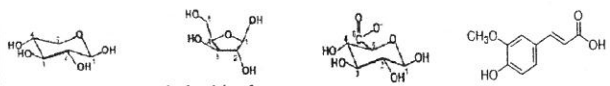


Figure adapted from: Obel et al. 2006



D-Xylopyranose Xyl*p* | L-Arabinofuran Ara*f* | D-Glucuronic Acid GlcA | Ferulic Acid

OBJECTIVES

SHORT TERM:

Establish a resource for correlating rice gene expression and cell wall content throughout development

Determine which if any putative feruloyl transferases correlate with accumulation of ferulic acid residues in the cell wall.

MEDIUM TERM:

Characterize knock down and over expression mutants of promising putative FTs toward gaining insight into the biological roles of ferulic acid in grass cell walls.

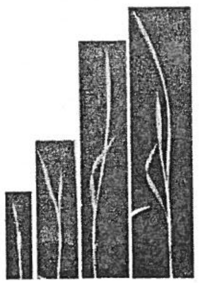
LONG TERM:

Use the knowledge gained to devise an informed strategy for improving grass feedstock digestability

Targeting a Clade in the Acyl CoA Transferase Family

- Mitchell *et al.* identified 12 rice loci from Pfam family PF02458, which is part of the CoA-acyl transferase superfamily as putative feruloyl transferases (FTs). We analyzed this family in rice and identified 8 additional closely related genes.
- Publicly available gene expression data from Jain *et al.* and others showed that 4 putative FT genes from 2 clades show high constitutive expression, but no obvious enrichment in tissues thought to be enriched for ferulic acid (not shown).
- **Conclusion: While the public gene expression data are useful, the organs and growth stages for which data are available do not address the question of which genes are expressed in expanding leaves. Further little data is available for rice on cell wall content throughout development.**

Establishing A Resource for Correlating Rice Gene Expression and Cell Wall Content



RNA resource at different developmental stages

Quantitative RT-PCR amplify with primers for the most highly expressed putative feruloyltransferases to see how these gene's transcripts change over development.

Cell wall analysis to determine monosaccharide composition and hydroxycinnamic acids and ferulic acids through development in Rice.

Unexpected Patterns of Ferulate Content

In contrast to the observations of Obel *et al.* (2002) for wheat, rice ferulic acid content is high in very young leaves and decreases with maturation. Young leaf levels of ferulate and *p*-coumarate are much higher in rice than wheat. Hydroxycinnamic acids are also high in lignified tissues.

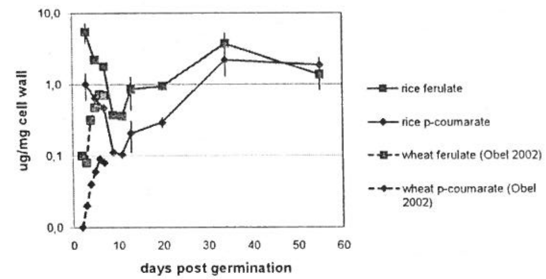


Figure. Leaf ferulic acid and p-coumaric acid throughout development for rice (this study) and wheat (Obel et al. 2002). Tissue samples were ground in liquid nitrogen and alcohol insoluble residue was isolated with repeated extraction with hot, aqueous ethanol. Samples were destarched and treated with NaOH prior to extraction with ethyl acetate. Ethyl soluble phenolics were seperated by HPLC on a C18 column.

Table. Ferulic acid and p-coumaric content of selected rice organs.

Sample	plant age (days post germ.)	Ferulic Acid (µg/mg)	*p*-Coumaric Acid (µg/mg)
Hard node and stem tissue	20	6.2 ± 0.4	7.4 ± 0.8
Very soft, immature emerging leaf	34	10 ± 1	1.9 ± 0.1
Long lvs (4,5,6 fm. bt), bottom 1/3rd	34	5.7 ± 0.4	3.4 ± 0.2
Long lvs, mid 1/3rd	34	3.3 ± 0.3	1.9 ± 0.1
Long lvs, top 1/3rd	34	2.2 ± 0.1	1.3 ± 0.1
Older, leaf sheath from lvs 1-4	34	6.5 ± 0.6	5.0 ± 1.2
Midvein	55	1.8 ± 0.3	2.3 ± 0.2
Leaf no midvein	55	0.9 ± 0.3	1.4 ± 0.1
Node	55	6.5 ± 1.0	14 ± 2
Stem	55	5.4 ± 0.3	11 ± 0.4
Lvs of large, senesced tillers	100	0.3 ± 0.2	1.2 ± 0.3
Sheaths of large senesced tiller	100	2.6 ± 0.6	4.8 ± 0.4
Stem of senesced tiller	100	5.1 ± 1.1	12 ± 2
Ligule and auricle	100	11 ± 1	7.1 ± 0.1

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